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The role of kynurenine and UV light in lens protein modification

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THE ROLE OF KYNURENINE AND UV LIGHT IN LENS PROTEIN MODIFICATION

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DOCTOR OF PHILOSOPHY

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By

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CERTIFICATION

I, Nicole Renee Parker, declare that this thesis, submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Nicole Renee Parker

8/7/05

In memory of Hossein,

By wisdom the LORD laid the earth's foundations,

by understanding he set the heavens in place;

by his knowledge the deeps were divided

and the clouds let drop the dew.

My son, preserve sound judgement and discernment,

do not let them out of your sight;

they will be life for you,

an ornament to grace your neck.

Let the favour of the Lord our God be upon us,

And establish the work of of our hands

Proverbs 19-22 & Psalm 90:17

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PUBLICATIONS

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LIST OF ABBREVIATIONS

ATP	adenosine triphosphate
ACN	acetonitrile
ACR	acrolein
AGE	advanced glycation end products
AHBG	4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid *O*-β-D-glucoside
Arg	arginine
ARN	age-related nuclear
Asn	asparagine
Asp	aspartic acid
Boc	butyloxycarbonyl
CEL	N^{ε}-carboxymethyl-L-lysine
CLP	calf lens protein
CML	N^{ε}-(carboxymethyl)-L-lysine
Cys	cysteine
D	dioptre
Da	dalton
D_2O	deuterium oxide
DTNB	5,5'-dithio-bis(2-nitrobenzoic acid)
DTT	dithiothreitol
di-Tyr	di-tyrosine
DOPA	3,4-dihydroxyphenylalanine
EDTA	ethylenediaminetetraacetic acid
Em	emission

EPR	electron paramagnetic resonance
ESI-MS	electrospray ionisation mass spectrometry
Ex	excitation
Gln	glutamine
GOLD	glyoxal lysine dimer
GR	glutathione reductase
GSH	glutathione
GSHPX	glutathione peroxidase
HOHICA	hexahydro-1H-indol-2-carboxylic acid
His	histidine
RP-HPLC	reversed-phase high performance liquid chromatography
H_2O_2	hydrogen peroxide
$OH^{\cdot}$	hydroxyl radical
OH^{-}	hydroxyl ion
4HNE	4-hydroxynonenal
IAEDANS	*N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine
IDO	indoleamine 2,3-dioxygenase
KAT	kynurenine aminotransferase
Kyn	kynurenine
Leu	leucine
Lys	lysine
MES	2-[*N*-morpholino]ethanesulfonic acid
Met	methionine
MIANS	6-(4'-maleimidyl-anilino)naphthalene-2-sulfonic acid
MG-H	methylglyoxal hydroimidazolone

MOLD	methylglyoxal lysine dimer
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NaN_3	sodium azide
NFK	L-*N*-formylkynurenine
NIR	near infrared
$OH^{\cdot}$	hydroxyl radical
$^1O_2$	singlet oxygen
$O_2^{\cdot -}$	superoxide radical anion
3-OHK	3-hydroxykynurenine
3-OHKG	3-hydroxykynurenine *O*-β-D-glucoside
Phe	phenylalanine
PMSF	phenylmethanesulfonyl fluoride
PSH	protein sulfhydryl
PSSC	protein-S-S-Cys
PSSG	mixed disulfide
PSSGC	protein-S-S-γ-glutamylcysteine
PSSP	protein disulfide
ROO.	peroxyl radical
ROOH	hydroperoxide
ROS	reactive oxygen species
RP-HPLC	reversed phase high performance liquid chromatography
SDS PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
TFA	trifluoroacetic acid
TNB	5-thio-2-nitrobenzoate anion

TLC	thin layer chromatography
TPCK	*N*-tosyl-L-phenylalanine chloromethyl ketone
Trp	tryptophan
Tyr	tyrosine
Val	valine
WISS	water-insoluble sonicate supernatant

ABSTRACT

Human lens proteins become progressively modified by tryptophan-derived UV filter compounds in an age-dependent manner. Kynurenine, for example, undergoes deamination at physiological pH, and the product binds covalently to the nucleophilic residues in protein *via* a Michael addition. Key sites of kynurenine modification in human lens proteins include cysteine, histidine and lysine residues.

The factors determining the levels of Kyn-amino acid adducts *in vivo* are not known. An aim of this study was to determine the rate of reaction of Kyn with nucleophilic amino acids (His, Cys and Lys) under physiological conditions (pH 7.2, 37˚C) and to evaluate the stability of the Kyn-amino acid adducts under these conditions. Kinetic and stability studies were performed using both free amino acids and modified calf lens protein, and reactions analysed by high performance liquid chromatography and mass spectrometry.

Kinetic studies using free amino acids revealed that Cys reacted with Kyn at approximately three times the rate of His and four times the rate of Lys. Kyn-*t*-Boc-His was found to be the most stable of all the Kyn-amino acid adducts under physiological conditions, followed by Kyn-*t*-Boc-Cys and Kyn-*t*-Boc-Lys.

Kyn-*t*-Boc-Lys and Kyn-Cys decomposed under physiological conditions, releasing deaminated Kyn. Kyn-Cys was the least stable of the Kyn-amino acid adducts. Incubation of Kyn-Cys in the presence of excess of *t*-Boc-L-His resulted in a decrease in Kyn-Cys and a corresponding increase in Kyn-*t*-Boc-His, due to the transfer of deaminated Kyn. Addition of a *t*-Boc group to the α-amino group of Cys increased the stability of the Kyn-Cys adduct by a factor of three. Oxidation of Kyn-*t*-Boc-Cys to a sulfoxide derivative decreased the stability by a factor of three.

Kinetic studies performed with calf lens protein incubated with Kyn showed that Cys was the preferred site of Kyn modification, followed by His and Lys, repectively. Following 14 days of incubation, Kyn-Cys was present at 12- and 17-fold greater levels than Kyn-His and Kyn-Lys. Protein-bound Kyn-Cys appeared more stable than the free Kyn-Cys adduct and was similar in stability to the free Kyn-*t*-Boc-Cys adduct. Under these conditions, protein-bound Kyn-His levels increased by 38%, whereas Kyn-Lys decreased by 28% with incubation time. Protein-bound Kyn-Lys was also relatively more stable compared the free Kyn-amino acid adduct.

Both the free amino acid and calf lens protein studies confirmed that Cys was the best nucleophile at physiological pH, followed by His and Lys. Kyn-His, however, was the most stable modification. The final pattern of Kyn-modification was shown to be a factor of amino acid reactivity and stability rather than amino acid abundance.

Another aim of this study was to explore the hypothesis that protein-bound Kyn is oxidised in the cataractous lens, as the levels of Kyn-His and Kyn-Lys decrease by a factor of 4 with increasing severity of age-related nuclear (ARN) cataract.

Model studies were performed in which Kyn-*t*-Boc-His and Kyn-*t*-Boc-Lys were incubated in the presence of an equimolar concentration of hydrogen peroxide. These studies showed that hydrogen peroxide did not affect the decomposition of either adduct under physiological conditions, suggesting that decomposition of these species in the cataractous lens may be the result of other factors, for example, stronger oxidising agents.

The final aim of this study was to investigate the photochemistry of protein-bound Kyn. Previous studies have shown that Kyn, when free in solution, is an inefficient sensitizer of oxidative damage. However, the photochemistry of protein-bound UV filter molecules has not been investigated and this may be of significance, especially for the older human lens, as a result of the decline in free Kyn and an increase in the bound form.

Lens proteins covalently modified with kynurenine were susceptible to photo-oxidation by wavelengths of light that penetrate the cornea (UVA light λ>305, >345 and >385 nm). These wavelengths were chosen because light in the 300-400 nm band are absorbed by the lens. Hydrogen peroxide and protein-bound peroxides were found to accumulate in a time-dependent manner after exposure to UV light (λ 305-385 nm), with shorter wavelength light generating more peroxides. Peroxide formation was accompanied by increases in the levels of protein-bound tyrosine oxidation products di-tyrosine and 3,4-dihydroxyphenylalanine, species known to be elevated in human cataract lens proteins. Experiments using D_2O, which enhances the lifetime of singlet oxygen, and azide, a potent scavenger of this species, are consistent with oxidation being mediated by singlet oxygen. These findings provide a mechanistic explanation of UV light-mediated protein oxidation in cataract lenses, and also rationalise the occurrence of age-related cataract in the nuclear region of the lens, as modification of lens protein by UV filters occurs primarily in this region.